20ml, the resulting crystals were filtered and washed with isopropanol / isopropyl ether, gave 4-Phenyl-4'- guanidinobenzoate hydrochloride 2.2g (yield 75%).
LC/MS=255(M+H)

4-(4-Biphenyl)-4'- guanidinomethylbenzoate hydrochloride

A suspension of 4-guanidinomethylbenzoic acid hydrochloride 2.3g(0.010mol), 4-phenylphenol 1.7g (0.010mol) and dicyclohexylcarbodimide 4.1g (0.020mol) in pyridine (150ml) was stirred at room temperature for 48hrs. After removal of insoluble materials by filtration, the filtrate was evaporated to dryness and residual solid was treated with 0.1N hydrochloric acid (50ml), washed with ether. The aqueous layer was concentrated to 20ml, the resulting crystals were filtered and washed with isopropanol / isopropyl ether, gave 4-(4-biphenyl)-4'- guanidinomethylbenzoate hydrochloride 2.5g (yield 65%).
LC/MS=346 (M+H)

4-(4-Biphenyl)-4'- guanidinobenzoate hydrochloride

A suspension of 4-guanidinobenzoic acid hydrochloride 2.2g(0.010mol), 4-phenylphenol 1.7g (0.010mol) and dicyclohexylcarbodimide 4.1g (0.020mol) in pyridine (150ml) was stirred at room temperature for 48hrs. After removal of insoluble materials by filtration, the filtrate was evaporated to dryness and residual solid was treated with 0.1N hydrochloric acid (50ml), washed with ether. The aqueous layer was concentrated to 20ml, the resulting crystals were filtered and washed with isopropanol / isopropyl ether, gave 4-(4-biphenyl)-4'- guanidinobenzoate hydrochloride 2.6g (yield 70%).
LC/MS=332 (M+H)

4-(4-Methylphenyl)-4'-guanidinomethylbenzoate hydrochloride

A suspension of 4-guanidinomethylbenzoic acid hydrochloride 2.3g (0.010mol), 4-methylphenol 1.1g (0.010mol) and dicyclohexyl- carbodimide 4.1g (0.020mol) in pyridine (150ml) was stirred at room temperature for 48hrs. After removal of insoluble materials by filtration, the filtrate was evaporated to dryness and residual solid was treated with 0.1N hydrochloric acid (50ml), washed with ether. The aqueous layer was concentrated to 20ml, the resulting crystals were filtered and washed with isopropanol /

isopropyl ether, gave 4-(4-methylphenyl) -4'- guanidinomethylbenzoate hydrochloride 2.4g (yield 75%).

LC/MS=284 (M+H)

4-(4-Methylphenyl)-4'-guanidinobenzoate hydrochloride

A suspension of 4-guanidinobenzoic acid hydrochloride 2.2g(0.010mol), 4-methylphenol 1.1g (0.010mol) and dicyclohexyl- carbodimide 4.1g (0.020mol) in pyridine (150ml) was stirred at room temperature for 48hrs. After removal of insoluble materials by filtration, the filtrate was evaporated to dryness and residual solid was treated with 0.1N hydrochloric acid (50ml), washed with ether. The aqueous layer was concentrated to 20ml, the resulting crystals were filtered and washed with isopropanol / isopropyl ether, gave 4-(4-methylphenyl) -4'- guanidinobenzoate hydrochloride 2.2g (yield 75%).

LC/MS=270 (M+H)

References and notes:

1. US Patent 4, 348,410
2. J.O.C. vol. 33 (1985) 652
3. Wenren et al Reactions of drugs synthesis published by chemical industry of China.
4. Chen fener et al Synthesis methods of organic drug published by Pharmaceutical science technology of China

Example 2. Activities of GMCHA derivatives

Different modifications of GMCHA were found to have differing inhibitory effects on the growth of *E. coli* (Table 1). For example, while the phenyl ester (PH01) derivative had an IC_{50} of >200 μM on *E. coli* growth, various modifications at the 4-methylpheny (PH02), 4-ethylphenyl (PH03), 4-tert-butylphenyl (PH04), and 4-biphenyl (BP01) decreased from >200, to 167, to 45, and to 26μM, respectively. Significantly, the effects of these compounds were not restricted to *E.coli*. (Irisawa *et al.*, *Biol. Pharm. Bull.*, 16:1211-1215 (1993); and Kato *et al.*, *J. Enzyme Inhibition*, 8:25-37 (1994)). The relative effects of individual members of this class of molecules remained the same whether the target cells were *E.coli, B. Subtilis, S. aureus* or *S. epidermidis* (Table 2). In

each case, the most effective compound remained the 4-biphenyl (BP01) derivative. Interestingly, the IC_{50} for a specific compound varied significantly for different bacterial species, with almost a two-log difference between those that were tested. For the 4-biphenyl ester (BP01), for example, it seemed that Staphylococcus was one log more sensitive than Bacillus, which was in turn one log more sensitive than Escherichia.

Table 1. Effects of GMCHA derivatives on E. coli Growth and Proteinase In Activity

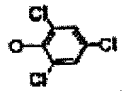
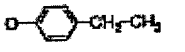
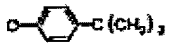
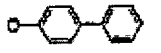
	COMPOUND	STRUCTURE	*E. coli* Growth IC_{50} (μM)	*E. coli* Proteinase In IC_{50} (μM)	Trypsin K_i (μM)
PH01	Phenyl		>200	>200	110
PH02	4-Methylphenyl		>200	>200	78
PH03	4-Ethylphenyl		167	>200	48
PH04	4-*tert*-Butylphenyl		45	38	64
PH05	2,4-Dichlorophenyl		92	62	46
PH06	2,4,6-Trichlorophenyl		44	35	273
BP01	4-Biphenyl		26	17	54
BP02	2-Biphenyl		74	83	187

Table 2. Effects of Various Aromatic Esters of GMCHA on the Growth of Different Bacterial Species

	COMPOUND	STRUCTURE	*E. coli* IC_{50}	*E. coli* IC_{100}	*B. subtilis* IC_{50}	*B. subtilis* IC_{100}	*S. aureus* IC_{50}	*S. aureus* IC_{100}	*S. epidermidis* IC_{50}	*S. epidermidis* IC_{100}
PH01	Phenyl		>200	>200	>200	>200	151	>200	128	>200
PH02	4-Methylphenyl		>200	>200	>200	>200	47	120	48	120
PH03	4-Ethylphenyl		167	>200	129	>200	15	50	14	50
PH04	4-*tert*-Butylphenyl		45	90	26	50	3.4	15	2.9	10
BP01	4-Biphenyl		26	40	4	10	0.6	2	0.4	1.5